

119929-1031/U.S. Patent Appl. No. 09/846727

SUBSTITUTE SHEET

each resonance is proportional to ^2H -enrichment at that position, so the spectrum provides a simple and direct readout of ^2H enrichment ratios.

[0038] It is important to point out that the ^2H NMR measurement is not influenced by the presence of tracer levels of ^{13}C in the glucose (or monoacetone glucose) molecule. Table 1 summarizes the relative contributions of glycogen, glycerol and PEP to glucose production as measured by deuterium NMR of monoacetone glucose derived from blood glucose. Fluxes through key pathways involving the TCA cycle were measured relative to flux through citrate synthase by analysis of carbon-13 NMR spectra from urinary acetaminophen glucuronide or urinary phenylacetylglutamine. The equation (eqn) used to calculate a given value is indicated.

TABLE 1

Sources of glucose (fraction from ^2H NMR of blood monoacetic glucose)			Flux ratios relative to citrate synthase (from ^{13}C NMR of urine acetaminophen glucuronide)			Flux ratios relative to citrate synthase (from ^{13}C NMR of urine phenylacetylglutamine)			
subject	glycogen (eqn 1)	glycerol (eqn 2)	PEP (eqn 3)	OAA \rightarrow PEP (eqn 4)	PEP \rightarrow pyruvate (eqn 5)	PEP \rightarrow glucose (eqn 6)	OAA \rightarrow PEP (eqn 7)	PEP \rightarrow pyruvate (eqn 8)	PEP \rightarrow glucose (eqn 9)
A	0.57	0.00	0.43	6.61	5.18	1.44	6.58	5.90	0.69
B	0.55	0.06	0.39	7.41	5.10	2.31	6.34	5.44	0.90
C	0.43	0.01	0.56	5.50	3.76	1.74	6.19	5.03	1.12
D	0.45	0.06	0.49	7.51	5.62	1.89	6.80	5.73	1.07
E	0.59	0.03	0.38	8.85	6.68	1.85	5.66	4.96	0.70
mean	0.52	0.03	0.45	7.11	5.27	1.85	6.31	5.41	0.90
s.d.	0.07	0.03	0.08	1.13	1.05	0.31	0.44	0.41	0.20

TCA cycle and gluconeogenic flux measurements from [$U-^{13}\text{C}_3$]propionate

incorporation into hexose and PAGN.

~~TCA cycle and gluconeogenic flux measurements from [$U-^{13}C_3$]propionate incorporation into hexose and PAGN~~

[0039] Relative anaplerotic, pyruvate recycling, and gluconeogenic fluxes can be obtained by a ^{13}C isotopomer analysis of plasma glucose, urinary glucuronide, or the glutamine fragment in urinary PAGN. The equations that describe these relationships are given by eqns. 4-9.

[0040] Figure 5 illustrates typical multiplets observed in the ^{13}C NMR spectrum of plasma glucose C 2β and urinary glucuronate C 5β of the same individual. The multiplet pattern arises from metabolism of [$U-^{13}C_3$]propionate at the level of the liver TCA cycle and is not affected by the presence of or metabolism of [$1,6-^{13}C_2$]glucose. The difference in signal-to-noise in these two spectra is largely due to the amount of urinary glucuronate in ~100-150 mL of urine compared to the amount of glucose in 10 mL of blood. Given that the multiplets in blood glucose C 2β and urinary glucuronate C 5β report identical flux values and the large differences in signal-to-noise of the spectra shown, relative flux values as reported by the glucuronate

GARDERE

attorneys and counselors • www.gardere.com

Fax

DATE: May 11, 2007

TO	COMPANY	PHONE NO.	FAX NO.
Jill Warden	USPTO	571-272-1267	571-273-1267

FROM Monique Vander Molen
DIRECT LINE 214-999-4330
DIRECT FAX 214-999-3330
CLIENT/MATTER NO. 119929-1031
TOTAL PAGES (including cover) 3

If you have any problems with this transmission, please call .

Is this for Service of Documents? No
Please indicate local time deadline:
Confirmation Requested: No

Re: U.S. Patent Application No.: 09/846727

Dear Supervising Examiner Warden:

Per your instructions delivered by voicemail on May 10, 2007, provided by facsimile are two substitute sheets for pages 22 and 23 of the specification with a new copy of Table 1 and corrections of two typographical errors in paragraph [0038]. Kindly confirm receipt.

Monique *Monique A. Vander Molen*

CERTIFICATE OF FACSIMILE TRANSMISSION

(37 CFR 1.6d)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited BY FACSIMILE transmission on the date shown below to Mail Stop Amendment, Commission for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, Washington, D.C. 20231 at 571-273-1267 Dated: May 11, 2007.

Monique A. Vander Molen (Typed name of person faxing paper)

Monique A. Vander Molen (Signature of person faxing paper)

NOTICE BY GARDERE WYNNE SEWELL LLP

The message herein contained or attached documents accompanying this facsimile contains information from the law firm of Gardere Wynne Sewell LLP which is confidential and/or privileged, or may contain attorney work product. The information is intended only for the use of the addressee named above. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of such facsimile and attachments is strictly prohibited, and may be unlawful. If you have received this facsimile in error, please destroy without disclosing the contents, and notify the sender immediately. Unintended transmission does not constitute waiver of the attorney-client privilege or any other privilege.

Unless expressly stated otherwise, nothing contained in such message should be construed as a digital or electronic signature, nor is it intended to reflect an intention to make an agreement by electronic means.

DALLAS 1788462v1

GARDERE WYNNE SEWELL LLP
1601 Elm Street, Suite 3000 Dallas, Texas 75201-4761 • 214.999.3000 Phone • 214.999.4667 Fax
Austin • Dallas • Houston • Mexico City • Washington, DC

05-11-07 15:18 From-GARDERE
+2149994176 T-415 P.01/03 F-865